Research Article

The Effects of UV Radiation on Chloroplast Clumping and Photosynthesis in the Seagrass *Halophila stipulacea* Grown under High-PAR Conditions

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Since potentially harmful ultraviolet radiation (UVR, 280–400 nm) and high photosynthetically active radiation (PAR, 400–700 nm) are present in the shallow waters of the Gulf of Aqaba where part of the seagrass *Halophila stipulacea*’s population thrives, we examined the effects of high PAR with and without UVR on its photosynthesis and midday chloroplast “clumping phenomenon” (Sharon and Beer 2008). It was found that midday clumping occurred only under high PAR in the presence of UVR, which resulted in a 44% reduction in the absorption cross section (or absorption factor, AF) of the leaves and, accordingly, a parallel lowering of midday electron transport rates (ETR). In addition, UVR had a direct effect on the photosynthetic apparatus by lowering quantum yields and, thus, ETRs, while pigment relations remained unaltered. We conclude that the potentially harmful effects of UVR and high PAR on the photosynthetic apparatus of *Halophila stipulacea* are mitigated by their activation of chloroplast clumping, which functions as a means of protecting most chloroplasts from high irradiances, including UVR.

1. Introduction

While the mechanisms underlying chloroplast movements in plants have been studied on the intracellular and molecular levels [1, 2] their ecophysiological role(s) is less understood. These movements are in many plants induced by blue light [3–8], but in a few studies it was suggested that also ultraviolet (UV), or “near to UV”, wavelengths could trigger chloroplast movements [9, 10]. Interestingly, Kondo et al. [11] reported that in many cases the chloroplast clumps are formed near or around the nuclei so as to protect them from high irradiances.

The only seagrass so far for which chloroplast movements have been described is *Halophila stipulacea* [12, 13]. In the latter work, it was found that chloroplast clumping occurred during midday only in plants growing under high irradiances (>450 μmol photons m⁻² s⁻¹), while lower-irradiance grown plants maintained their chloroplasts evenly dispersed during the day. It was then suggested that the chloroplast clumping during midday may have a protective role against high irradiances.

In addition to high photosynthetically active radiation (PAR, 400–700 nm), coastal waters, especially clear tropical waters, also feature high levels of ultraviolet radiation (UVR, 280–400 nm) in their upper layers ([14, 15] and this study). Various marine plants respond to such high UVR levels by producing mycosporine-like amino acids (MAA, [16, 17]) and other UV absorbing compounds (i.e., flavones and flavones glycosides in seagrasses, [18–20]), changing morphologies [21], changing pigment contents [17, 22], downregulation of photosystem II (PSII, [23]), or shading by carbonate layers [24]. Also epiphytes may decrease the UVR (as well as PAR) levels reaching the leaves [25, 26]. Since many seagrasses thrive in clear tropical shallow waters, it may be that the midday chloroplast clumping found in at least one of them (*Halophila stipulacea*) has a protective role against UVR (in addition to high PAR as suggested by Sharon and Beer [13]). In light of this possibility, we investigated the role...
of UVR in mediating chloroplast movements in this seagrass and the effects of UVR on some of its photosynthetic traits in plants acclimated to high irradiances.

2. Materials and Methods

Whole plants (including roots and rhizomes) of *Halophila stipulacea*, together with surrounding sediments, were collected at 20 m depth from the densest part of a monospecific meadow (extending from 7 to 33 m at the time of this study; the tidal range is <40 cm) located ca. 200 m south of the Inter-University Institute (IUI) in Eilat, Gulf of Aqaba, northern Red Sea (29°30'N, 34°54'E) during March and April, 2007. These plants were grown in cups containing their natural fine sandy sediment on an outdoor water table (a table with a 20 cm margin, forming a shallow container) through which natural seawater at 21°C was flowing. Thus, the leaves were submerged 5–10 cm under the water surface. The water table was shaded by a neutral-density net allowing 50% of the natural sunlight to reach the plants. Typical irradiances under the net were ~850 μmol photons m⁻² s⁻¹ during midday. The light under the shading net was further screened either through UV filters (supplied by the GKSS Forschungszenrum, Germany) placed just above the water surface over some of the plants or through control filters that transmitted UVR (from the same supplier). Both filters reduced PAR by ~20%, while the UV filter reduced wavelengths <400 nm by >80% (see Table 1 and Figure 2). The wavelength-dependent transmittance properties of the filters were verified using a miniature-fibre optical spectrometer (USB 2000, Ocean Optics, USA). All plant measurements were done after 14 days of acclimation to the water-table conditions. This time was found to be enough for the plants to feature diurnal chloroplast clumping under the control filters and was also found to be sufficient for the acclimation of several photosynthetic parameters to the chosen light regimes (see also [27]).

The solar irradiance (PAR and UVR) reaching the water surface was measured by a CM11B (Kipp & Zonen, The Netherlands) sensor at the IUI pier (displayed also at http://www.meteo-tech.co.il/eilat-yam/eilat_daily.asp). Spectral radiation measurements were done using a profile reflectance radiometer (PRR-800, BioSpherical Instruments Inc., USA) equipped with both spectral channel (300–875 nm, μW cm⁻² nm⁻¹) and PAR (400–700 nm, μmol photons m⁻² s⁻¹) sensors. Underwater spectral measurements of downwelling UVR, PAR, and infrared radiation were also done using the PRR-800, which was lowered from a boat offshore the IUI using a free-fall system (cf. [28]) in order to avoid boat shading and to keep the instrument in a vertical posture.

Chloroplast clumping was verified through microscopic observations on live leaves within 2 min of collection (using a Nikon YS100, Japan). The absorption cross sections (or absorption factor, AF) of the leaves (i.e., the fraction of incident irradiance absorbed by the photosynthetic pigments of the leaves) were estimated along the day by measuring the PAR above and below a submerged leaf with the Diving-PAM’s quantum sensor (while subtracting the absorption of nonchlorophyllous pigments, see [13]). Chlorophyll contents of the leaves were determined after extraction in dimethyl formamide for 24 h in darkness. Absorbance was then measured spectrophotometrically according to Moran [29], and chlorophyll a and b contents were calculated on a leaf area basis. Total carotenoids were measured by their absorption maxima at 480 and 510 nm according to Gradinaru et al. [30]. Absorption spectra (300–400 nm) with special attention to absorption at 340 and 350 nm of the UV absorbing pigments (flavones and flavone-glycosides [20]) were measured after extraction in methanol using an Ultraspec 2100 Pro (Amersham Biosciences, UK) spectrophotometer.

Chlorophyll fluorescence was measured by pulse-amplitude-modulated (PAM) fluorometry using a Diving-PAM (Walz, Germany). Effective quantum yield (ΔF/ΔFm’) measurements were carried out throughout the day using a leaf-distance clip (Walz, Germany), and photosynthetic electron transport rates (ETR) were calculated as ΔF/ΔFm’ × PAR × AF × 0.5; PAR was measured with the Diving-PAM’s quantum
sensor calibrated against a quantum-sensor-equipped Li-250A light meter (LiCor, USA).

3. Results

The midday underwater spectra (300–800 nm) from two depths and the integrated total PAR (400–700 nm) and UVR (300–400 nm) at those depths, as well as on the water table under the control or UV filters, are shown in Figure 1 and Table 1, respectively. The spectra (Figure 1) reveal high UVR values at 6 m depth that could hardly be detected at 30 m. Similarly, there were measurable amounts of red light (650–750 nm) available at 6 m but not at 30 m. In all, approximately 45% and 5% of surface PAR were present at 6 and 30 m depths, respectively, while the corresponding percentages for UVR were 50% and 3% (Table 1). The PAR measured under the filters on the water table was 45% of that of full sunlight while UVR was 41% and 7.4% under the control and UV filters, respectively (see Figure 2 for the spectral transmission of these filters). Unlike UVR, there was no reduction in red wavelengths by the UV filter (Figure 2).

The epidermal leaf cells of plants grown on the water table under control-filtered conditions featured midday clumping of chloroplasts, followed by their dispersal during the evening, while no such chloroplast movements could be detected in the UV-filtered plants (Figure 3). While peaking at midday, chloroplast aggregation and dispersal were observed between 09:00 and 15:00 and were paralleled by decreasing and increasing AF values, respectively. Because the only difference between the treatments was the presence and virtual absence of UVR, it is concluded that the UVR present for the control-filtered plants caused the chloroplast movements at the given PAR that resulted in their midday clumping. More precisely, it is likely that the wavelengths between 280 and 380 nm caused the clumping since these wavelengths were the absolute cut-off points for the control and UV filters, respectively. In comparison, also leaves of plants growing at 6 m depth clumped their chloroplasts during midday while those at 30 m did not ([31] and personal observations). These chloroplast movements resulted in a significant change (44%, $P < 0.0001$, t-test, $n = 10$) in the AF of the control-filter-grown plant leaves from early morning to midday while the UV-filtered plants did not show any significant differences in AF during the day (Figure 4, $P > 0.05$, t-test, $n = 10$); the AF values of the plants growing under the control filters were significantly lower ($P < 0.01$, repeated measure ANOVA, $n = 10$) than those of the UV-filtered plants between 10:00–14:00.

Chlorophyll a + b contents showed no significant differences ($P > 0.05$, t-test, $n = 8$) between the UV- and control-filtered water-table plants after 14 days: Chlorophyll a contents were $2.3 \pm 0.17$ and $2.6 \pm 0.27$ mg cm$^{-2}$ for the control- and UV-filtered plants, respectively, while chlorophyll b contents were $0.3 \pm 0.01$ mg cm$^{-2}$ in both control- and UV-screened plants. Also carotenoid-absorption spectral peaks (at 480 and 510 nm) showed no significant difference ($P > 0.05$, t-test, $n = 8$) between the two types of water-table grown plants after 14 days of acclimation. Similarly, neither the absorption spectra in the region where flavones and flavones glycosides absorb (340–350 nm), nor the peak maxima of anthocyanin (290 nm), showed significant differences ($P > 0.05$, t-test, $n = 8$) between treatments with or without UVR.
Photosynthetic ETRs of the plants grown on the water table with or without UV screening are presented in Figure 5. Since these ETRs were calculated according to their respective AF values during the various times of the day (see Figure 4, cf. [13]), it follows that the differences in ETRs between control- and UV-filtered plants are partly due to the differences in AFs (here termed an “indirect” UVR effect). The maximum ETR reached at around midday by the UVR-protected plants coincided with the highest PAR reaching the water table while the ETRs for the control plants levelled off in the morning after ca. 9 o’clock. This, as well as the fact that the midday decrease in ETR in the control-filtered plants was more than the 44% that could be accounted for by the decrease in AF, indicates an excessive decrease of effective photosynthetic quantum yields by UVR under high PAR during the high-irradiance hours of the day. Indeed, ΔF/Fm’ values were significantly lower (P < 0.05, t-test, n = 8) in the control-filtered than the UV-filtered plants from around 11:30 and throughout the afternoon (Figure 6), confirming a higher downregulation of PSII in the former as caused by UVR with high PAR (here termed a “direct” UVR effect).

4. Discussion

In a previous study, we suggested that the midday chloroplast clumping observed in Halophila stipulacea had a photoprotective role against high PAR during midday [13]. Here, we found that it is UVR that triggers the diurnal chloroplast movements that cause their midday clumping under high-PAR conditions. This was realised at midday UVR values of ca. 2000 µW m⁻² on the water table (while filtering out UV prevented clumping). In nature, at depths where clumping does not occur (below ca. 10 m, at PARs <400 µmol photons m⁻² s⁻¹), reduced UVR is usually accompanied with low PAR, but the UVR/PAR ratio may also change (here it was reduced by 46% from 6 to 30 m). Thus, it is possible that also the UVR/PAR ratio has an effect on the clumping phenomenon in shallow waters, but this was not quantified here. While the surface irradiance characteristics of the region where this study was conducted (northern Red Sea) are among the highest in the world [32], we were intrigued by finding high UVR doses similar to those on the shaded water table also in situ at 6 m depth. Indeed, midday chloroplast clumping was observed at that depth [31]. Thus, apparently, the successful adaptation of this plant to shallow waters (~6 m at the site studied) is partly based on its ability to reduce the absorption cross section (or AF) via chloroplasts movements, apparently resulting in protection from high irradiances, including UVR. In addition to shading one another, the clumped chloroplasts could protect the nucleus and its genetic material from excess UV (such as suggested by Kondo et al. [11]).
While it was found in an earlier work that PAR correlated negatively with chlorophyll concentrations [13], manipulating UVR under constant PAR did not cause any changes in chlorophyll nor carotenoid contents in *Halophila stipulacea*. Supportively, also another *Halophila* species (*Halophila johnsonii*) did not show differences in absorption peak maxima for any photosynthetic and UV-protecting pigments when acclimated to various PAR and UV treatments [19] (but did increase its flavone and flavone-glycoside contents as a response to increased salinity, [33]). The apparent failure of *Halophila stipulacea* to induce the formation of UV-protecting substances stands in contrast to other tropical shallow-growing seagrasses [20, 22], corals [34], microalgae [17], and macroalgae [35], many of which do protect their tissues from UVR by the formation of UV-absorbing pigments. As based on our findings, apparently *Halophila stipulacea* utilizes chloroplast clumping instead of generating UV-blocking compounds in order to protect itself from high UVR and PAR. This mechanism may also be present in *Halophila ovalis*, which was found to produce less UV-blocking pigments than other genera [22], but this still needs to be proven.

While the reduction in midday ETRs was largely an indirect effect of UVR via the decreased AF values caused by the clumping of chloroplasts, UVR also affected photosynthesis directly by reducing quantum yields. The daily reduction in effective quantum yield can be seen as a dynamic form of photoinhibition (or downregulation of PSII) since values in the evening returned to morning values. One mechanism that could explain this midday reduction in effective quantum yields is the operation of the xanthophyll cycle, in which excess light energy is dissipated as heat [36]. Also, reaction centre repair via gene activation (e.g., D1-repair genes, [37]) can reduce photodamage and can be measured as a reversible diurnal damage. In addition, but to a lesser degree than the effective quantum yield, the maximal quantum yield (Fv/Fm) was also reduced by UVR with the acclimation time to the water-table conditions (data not shown). Since Fv/Fm is measured in predawn darkness, this decrease reflects an incomplete recovery from high irradiances that might indicate some chronic damage in the PSII repair mechanism. Thus, as part of the more general negative effects of UVR on phytoplankton [15] and other marine photosynthetic organisms [38], the chloroplast-based photosynthetic apparatus of some seagrasses is also influenced by UVR or high PAR with UVR. Perhaps logically, this radiation also activates mechanisms such as chloroplast clumping and a dynamic downregulation of photosystem II that protect at least *Halophila stipulacea* against it.

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**References**


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